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Endothelial 5-HT receptors mediate relaxation of porcine pulmonary arteries in response to ergotamine and dihydroergotamine

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1 The aim of the present study was to investigate whether antimigraine ergot compounds may act at endothelial 5-hydroxytryptamine (5-HT) receptors which trigger the release of endothelium-derived relaxing factor (EDRF). Changes in tone of porcine isolated pulmonary arteries were measured isometrically. The integrity of the endothelium was assessed by the bradykinin-induced relaxation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 3 μM)-precontracted vessels.

2 The ergot derivatives ergotamine, dihydroergotamine (DHE) and dihydroergocristine, as well as 5-HT and (±)-α-methyl-5-HT, elicited a reversible endothelium-dependent relaxation of $PGF_{2\alpha}$-precontracted arterial ring segments. The relaxation to both ergotamine and 5-HT was associated with an increase in cyclic GMP. After pretreatment of the vessels with N^G-nitro-L-arginine methyl ester (200 μM), or removal of endothelium by mechanical rubbing, the relaxant responses were abolished.

3 The mean pEC_{50} values for relaxant responses followed the order: (±)-α-methyl-5-HT (8.80) > 5-HT (8.75) > ergotamine (8.17) > DHE (7.70) > 5-carboxamidotryptamine (7.62) > dihydroergocristine (7.17).

4 The relaxant effects of both ergotamine and dihydroergotamine were resistant to block by indomethacin (3 μM), prazosin (1 μM) and ketanserin (1 μM). However, the ergotamine-induced relaxation was highly susceptible to block by pizotifen ($pA_2 = 8.23$), norclozapine ($pA_2 = 8.20$), methiothepin ($-\log IC_{50} = 7.26$), rauwolscine ($pA_2 = 7.24$) and mesulergine ($pA_2 = 6.64$). Each antagonist inhibited the relaxant responses to (±)-α-methyl-5-HT in the same manner with similar potency as that determined against ergotamine.

5 Recently, mRNA transcripts for $5\text{-}HT_{1D\beta}$ and $5\text{-}HT_{2B}$ receptors have been demonstrated in porcine pulmonary arteries. The rank order of potencies of agonists and antagonists in the present study suggests that the relaxant responses to 5-HT and ergot derivatives are mediated through activation of endothelial 5-HT receptors which are similar to the $5\text{-}HT_{2B}$ receptor subtypes.

Keywords: Porcine pulmonary arteries; endothelium-dependent relaxation; 5-HT; ergotamine; dihydroergotamine; 5-HT antagonists; $5\text{-}HT_{2B}$-receptors

Introduction

Ergot alkaloids such as ergotamine and dihydroergotamine (DHE) are widely used to treat migraine attacks and it has been suggested that their therapeutic efficacy is due to a selective vasoconstrictor action in the carotid vascular bed (Ferrari & Saxena, 1993). The vasoconstrictor effect of DHE is due, in part, to activation of 5-hydroxytryptamine (5-HT) receptors (Müller-Schweinitzer, 1984; Glusa & Markwardt, 1984; Müller *et al.*, 1988). Moreover, venoconstrictor responses to dihydroergocristine and DHE of canine saphenous veins are mediated through $5\text{-}HT_1$-like receptors (Müller-Schweinitzer, 1990). Radioligand binding studies in brain membranes have shown that ergotamine and DHE possess high affinity for several 5-HT receptor subtypes such as $5\text{-}HT_{1A}$, $5\text{-}HT_{1D}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$, 5-ht_{5-7} (Hoyer 1989; Hoyer & Schoeffter, 1991; Hoyer *et al.*, 1994; Peroutka, 1994). In piglet choroid plexus, ergotamine and DHE proved to be potent agonists at $5\text{-}HT_{2C}$ receptors inducing increases in inositol phosphates (Brown *et al.*, 1991). This is of special interest since it has been hypothesised that $5\text{-}HT_{2B}/5\text{-}HT_{2C}$ receptors are probably involved in the initiation of migraine (Kalkman, 1994; Fozard & Kalkman, 1994). In addition, recent studies suggest that the release of nitric oxide from endothelial cells might be an important trigger for migraine. (Olesen *et al.*, 1994). In this context it was of interest to determine whether the antimigraine drugs ergotamine and DHE may also stimulate endothelial receptors and elicit vasodilatation by release of EDRF.

In previous studies we have shown that in precontracted porcine pulmonary arteries 5-HT and other related agonists cause an endothelium-dependent relaxation via release of EDRF (Glusa & Richter, 1993). These endothelial 5-HT receptors exhibit some similarities to the $5\text{-}HT_{2B}/5\text{-}HT_{2C}$ subtypes (Glusa & Richter, 1993; Bodelsson *et al.*, 1993, Ellis *et al.*, 1995). In the present study various antagonists such as ketanserin, pizotifen, mesulergine, methiothepin (5-HT antagonists) prazosin (α_1-blocker), rauwolscine (α_2-adrenoceptor blocker) and norclozapine (an atypical antidepressant with affinity for $5\text{-}HT_{2C}$ and 5-ht_6-receptors, Kuoppamäki *et al.*, 1993) were used to characterize the receptor subtype(s) mediating the vasodilatation induced by ergot compounds and 5-HT agonists.

Methods

Measurement of relaxant and contractile effects

Pig lungs were obtained from the slaughter-house. Small branches of the pulmonary artery were carefully dissected, cleaned of adhering parenchyma and connective tissue and cut into rings of 2–3 mm in length. Each ring was mounted on L-shaped platinum hooks and placed in a 10 ml organ bath containing Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11; pH 7.4, 37°C) gassed continuously with a mixture of 95% O_2 and 5%CO_2. Each vascular ring was connected to an isometric force transducer (Hugo Sachs

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Elektronik, March, Germany) and changes in tension were recorded continuously. A passive resting tension of 20 mN was maintained throughout the experiments. During an initial stabilisation period of 60 min, the bathing medium was changed every 20 min and the tension repeatedly readjusted to 20 mN. The tissues were contracted at 45 min intervals once with KCl (30 mM) and 3 times with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 3 μM), a concentration producing about 50–60% of maximum response, until the effect was reproducible. Functional integrity of the endothelium was determined by relaxation of $PGF_{2\alpha}$-contracted vessels in response to addition of bradykinin (10 nM). This relaxation was absent after removal of the endothelium by gentle rubbing of the intimal surface of the rings with a roughened plastic stick. Endothelium-dependent relaxant responses were studied on $PGF_{2\alpha}$-precontracted vessels. With 5-HT, α-methyl-5-HT and ergotamine dose-relaxant response curves were determined by cumulative addition, the concentration in the organ bath being increased when steady-state relaxation to the previous concentration was achieved. Concentration-response curves for both dihydrogenated ergot derivatives were constructed from the mean responses to single concentrations. This method was employed, since it is known that many tissues respond only to the first concentration of these drugs and the cumulative concentration-response technique cannot be applied (Müller-Schweinitzer, 1990). The relaxation was expressed as the percentage of $PGF_{2\alpha}$-induced contraction before addition of the agonists. Antagonists were added 25 min before the agonists.

Determination of cyclic GMP

When, in $PGF_{2\alpha}$-precontracted arterial rings, the relaxant effect in response to the agonists had reached near-maximum (usually after 2 min), the vessels were rapidly removed from the organ bath and frozen in liquid nitrogen. The frozen samples were powdered by means of a dismembrator and then treated with 0.5 ml distilled water and 0.5 ml of 10% $HClO_4$ at 4°C for 60 min. After centrifugation the pellet was used for protein determination with bovine serum albumin as standard. EDTA (10 mM; 0.15 ml) was added to 0.6 ml supernatant and neutralized with 0.45 ml of a mixture of freon/trioctylamine. Centrifugation of the samples at 350 *xg* at 4°C for 2 min yielded three phases. From the aqueous upper phases 0.4 ml was lyophilized and dissolved in 0.1 ml buffer pH 7.4 for radioimmunoassay. The results were expressed as pmol of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) formed per mg protein.

Drugs

The following substances were used: 5-HT (5-hydroxytryptamine creatinine sulphate, serotonin), indomethacin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), bradykinin triacetate (Serva, Heidelberg, Germany), rauwolscine (Roth, Karlsruhe, Germany), ketanserin tartrate, methiothepin mesylate, ergotamine tartrate, prazosin hydrochloride, dihydroergotamine methanesulphonate (DHE), dihydroergocristine methanesulphonate, mesulergine hydrochloride, normethylclozapine, (±)-α-methyl-5-HT maleate, 5-carboxamidotryptamine maleate (5-CT) (Research Biochemicals International, Natick, MA, U.S.A.); pizotifen (Sandoz, Basel, Switzerland); N^G-nitro-L-arginine methyl ester (L-NAME, Sigma, Deisenhofen, Germany), [^{3}H]-cyclic GMP-radioimmunoassay (Amersham Buchler, Braunschweig, Germany).

Data analysis

Concentration-response curves were analyzed by use of non-linear, iterative curve-fitting to a three parameter logistic equation of the form: $E=(E_{max}[A]^n)/([A]^n+[A_{50}]^n)$ (Origin: Microcal Software, Inc.) and E_{max} (maximal effects) and pEC_{50} values (negative logarithm of the molar agonist concentration producing 50% of E_{max}) were derived from this analysis. For antagonists apparent pA_2 values were calculated according to the equation $pA_2=pA_x+\log(x-1)$, where x is the ratio of the concentrations of the agonist producing 50% of maximal response of the control curve in the presence and absence of the antagonist, and pA_x is the negative logarithm of the molar concentration of the antagonist used. For unsurmountable antagonism $-\log IC_{50}$ values (negative logarithm of the molar concentration of the antagonist which reduces the maximal effect of the agonist by 50%) were calculated according to van Rossum (1963). Data are presented as means ± s.e.mean. Statistical analysis was performed by Student's unpaired *t* test and the non-parametric Mann-Whitney test. Differences were considered statistically significant at $P<0.05$.

Results

Endothelium-dependent relaxation induced by 5-HT and ergot derivatives

In porcine pulmonary arteries with intact endothelium, the contractile response to $PGF_{2\alpha}$ (3 μM) amounted to 23.6 ± 1.0 mN ($n=45$). Bradykinin (10 nM) relaxed the precontracted vessels by 87 ± 4% ($n=40$) (Figure 1). The addition of ergotamine, DHE or dihydroergocristine to precontracted vessels resulted in a concentration-dependent reversible relaxation. Examples of representative experiments are shown in Figure 1. The relaxant effect of ergotamine reached its maximum at concentrations between 0.1 and 1 μM. At higher concentrations no further relaxation was observed, and instead tissue tension increased. Blockade of prostaglandin synthesis by indomethacin (3 μM) did not change the ergotamine- or 5-HT-induced relaxation. However, mechanical removal of the endothelium or pretreatment of the vessels with L-NAME (200 μM) attenuated the relaxant effect (Figure 1).

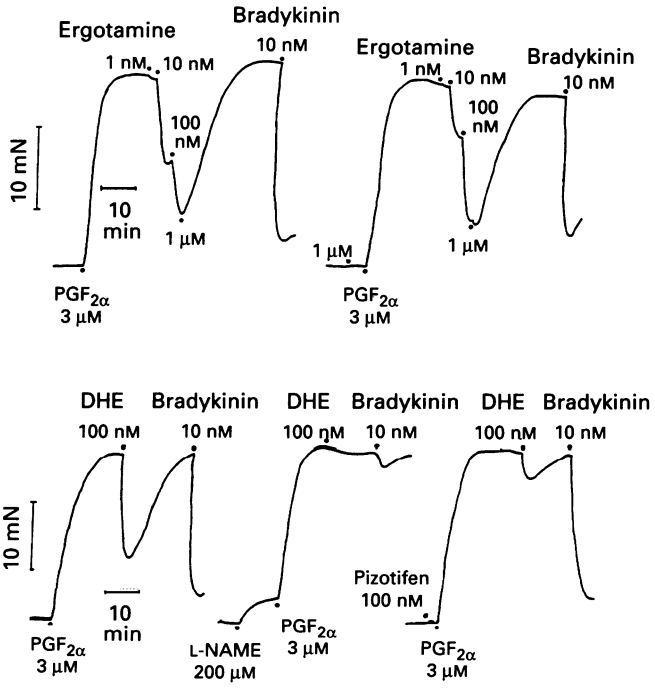

Figure 1 Representative tracings showing endothelium-dependent relaxation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-precontracted porcine pulmonary arteries induced by ergotamine, dihydroergotamine (DHE) and bradykinin. Preincubation with ketanserin did not inhibit the relaxant response to ergotamine while pretreatment with L-NAME (N^G-nitro-L-arginine methyl ester) or pizotifen attenuated the relaxant response to DHE.

To investigate whether ergotamine-induced relaxation was mediated through nitric oxide, cyclic GMP concentrations in the vascular tissues were determined. During stimulation of arterial rings with $PGF_{2\alpha}$ the basal cyclic GMP concentration amounted to 0.52 pmol mg^{-1} protein. This was significantly ($P<0.05$) diminished to 0.12 pmol mg^{-1} protein when the tissues were pretreated with L-NAME (200 μM). On the other hand, compared to $PGF_{2\alpha}$-stimulated control rings bradykinin (10 nM), 5-HT (0.1 μM) and ergotamine (0.1 μM) significantly increased the vascular cyclic GMP concentrations (Table 1).

Concentration-response relationships for relaxant responses to the agonists tested are shown in Figure 2. As indicated by the pEC_{50}-values (Table 2), (±)-α-methyl-5-HT and 5-HT were three to four times more potent than ergotamine and about ten times more potent than DHE. While 5-CT was about equipotent, dihydroergocristine proved to be three times less potent than DHE.

In endothelium-denuded vessels at resting tension, i.e. in the absence of $PGF_{2\alpha}$, contractile responses to 5-HT, ergotamine and DHE were very weak. Concentrations of more than 0.1 μM were required to induce contraction with a maximum of not more than 30% compared to the preceding $PGF_{2\alpha}$-induced contraction.

Table 1 Formation of cyclic cGMP in $PGF_{2\alpha}$-precontracted porcine pulmonary arteries before and after relaxation induced by bradykinin, 5-HT or ergotamine

Relaxant agent	*(n)*	*cyclic GMP (pmol mg^{-1} protein)*
Control (no relaxation)	(4)	0.52±0.15
Bradykinin (10 nM)	(7)	1.97±0.30*
5-HT (0.1 μM)	(8)	1.38±0.12*
Ergotamine (0.1 μM)	(8)	1.31±0.22*

Values are means±s.e. mean from n separate experiments. *$P<0.05$ significantly different from control value.

Table 2 pEC_{50} values and maximum endothelium relaxant responses to 5-HT and ergot derivatives of $PGF_{2\alpha}$-precontracted porcine pulmonary arteries

Agonist	n	*pEC_{50}*	*Maximum relaxation[1]*
(±)-α-Methyl-5-HT	25	8.80±0.04	91±2%
5-HT	20	8.75±0.04	89±2%
Ergotamine	18	8.17±0.07	73±8%
Dihydroergotamine	9	7.70±0.11	70±7%
5-Carboxamidotryptamine	5	7.62±0.14	88±5%
Dihydroergocristine	4	7.17±0.18	56±7%

Values are mean±s.e.mean of n separate experiments. [1]Percentage by which the arteries were relaxed related to the $PGF_{2\alpha}$-induced contraction.

Antagonism of endothelium-dependent relaxation

The α_2-adrenoceptor blocker rauwolscine (1 μM) inhibited relaxations induced by ergotamine and (±)-α-methyl-5-HT (Table 3). In contrast, both the α_1-adrenoceptor blocker prazosin (1 μM) and the 5-HT_{2A} receptor antagonist ketanserin failed to antagonize the relaxant effects of these agonists (pA_2 values $\leqslant 6$). Among the other antagonists tested pizotifen was the most potent inhibitory compound causing surmountable antagonism of the concentration-response curve for ergotamine (Figure 3b). The mixed 5-HT_1/5-HT_2 receptor antagonist methiothepin caused a parallel shift to the right of the ergotamine curve when used at 0.01 μM and attenuated the relaxant effects of ergotamine in an unsurmountable manner when used at ten times higher concentrations (Figure 3a). Norclozapine which has been described as a 5-HT_{2C} receptor antagonist (Kuoppamäki *et al.*, 1993), exhibited strong antagonistic potency when tested against ergotamine and 5-HT agonists, producing a shift to the right of the concentration-response curves of these agonists. The relaxant effect of ergotamine was also antagonized by mesulergine. Each antagonist tested inhibited the effects of both ergotamine and (±)-α-methyl-5-HT with similar potency (Table 3).

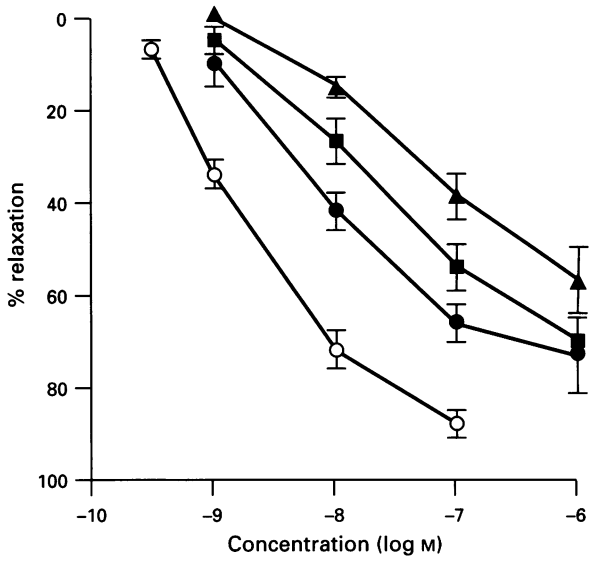

Figure 2 Concentration-response relationships for endothelium-dependent relaxant effects induced by 5-hydroxytryptamine (○, $n=15$), ergotamine (●, $n=14$), dihydroergotamine (■, $n=6$) and dihydroergocristine (▲, $n=4$). The effects are expressed as percentages of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 3 μM)-induced contraction. Means±s.e.mean (vertical lines) are shown.

Table 3 Inhibition of ergotamine- and (±)-α-methyl-5-HT-induced relaxation of $PGF_{2\alpha}$-precontracted porcine pulmonary arteries

Antagonist		*Ergotamine*	n	*(±)-α-Methyl-5-HT*	(n)
Ketanserin	pA_2	6.04±0.09	(8)	5.60±0.26	(4)
Pizotifen	pA_2	8.23±0.13	(12)	8.39±0.09	(10)
Methiothepin	$-\log IC_{50}$	7.26±0.31	(5)	7.66±0.20[1]	(8)
Mesulergine	pA_2	6.64±0.14	(7)	6.80±0.11[1]	(10)
Rauwolscine	pA_2	7.24±0.23	(7)	7.34±0.16	(5)
Norclozapine	pA_2	8.20±0.18	(6)	8.05±0.07	(15)

Values are mean±s.e.mean of n separate experiments. [1]Values from Glusa & Richter (1993).

When the antagonism of DHE-induced relaxation was investigated only one submaximal concentration (0.1 μM) of DHE was used. Pizotifen (0.1 μM) (Figure 1) and norclozapine (0.1 μM) suppressed the DHE effect by more than 80% while rauwolscine and ketanserin (1 μM each) diminished the DHE-induced relaxation by 50% and 20%, respectively.

Apart from its relaxant agonist activity ergotamine was able to antagonize the vascular effects of 5-HT. When ergotamine (10 nM) was added to $PGF_{2\alpha}$-precontracted vessels and if 5-HT was added cumulatively after the relaxant response (about 15 min later), the relaxant response to 5-HT was markedly attenuated (not shown).

Discussion

The results of the present experiments provide evidence that ergot alkaloids such as ergotamine, DHE and dihydroergocristine are able to elicit endothelium-dependent relaxations of $PGF_{2\alpha}$-precontracted vessels. Ergotamine was only three times less potent than 5-HT and its maximum effect amounted to about 80% of that of 5-HT (Table 2). On the other hand, the 5-HT-induced relaxation was markedly diminished in the presence of ergotamine (10 nM), though its relaxant effect had already disappeared, supporting the assumption that the ergotamine effect was mediated through activation of an endothial 5-HT receptor.

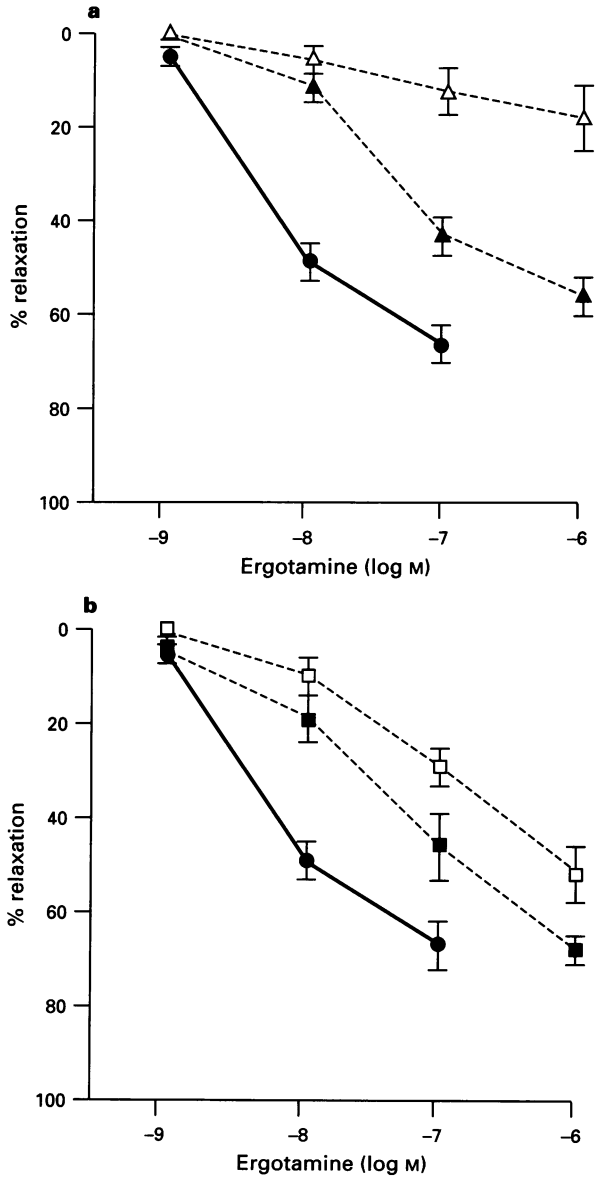

Figure 3 Concentration-response relationships for ergotamine-induced relaxant response of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 3 μM)-precontracted porcine pulmonary arteries. Control (●; solid line) and after preincubation with methiothepin (a; △, 0.1 μM; ▲, 0.01 μM) and pizotifen (b; □, 0.1 μM; ■, 0.03 μM). The effects are expressed as percentages of $PGF_{2\alpha}$ (3 μM)-induced contraction. Means ± s.e.mean (vertical lines) from 3–6 individual experiments.

Contractile responses of unstimulated endothelium-denuded vessels to ergotamine or DHE were observed only at higher concentrations (0.1 μM and at 10 μM), but these amounted to no more than 30% of the amplitude of the preceding $PGF_{2\alpha}$-contraction. The same was true of 5-HT. It therefore seems unlikely that relaxant responses to the ergots were influenced by an opposing contractile response at low drug concentrations. However, the biphasic response-curves to DHE and ergotamine might indeed be explained by a lower potency, direct contractile action on the vascular smooth muscle.

The relaxant effects of ergot compounds were due to an indirect mechanism which was absent in endothelium-denuded vessels. Furthermore, indomethacin did not impair the relaxant response thereby excluding the involvement of prostaglandins. These observations suggest that the ergot alkaloids might stimulate an endothelial receptor, known to increase inositol phosphates and cytosolic calcium which in turn leads to activation of the NO-synthase. Indeed, both ergotamine and 5-HT caused significant increases in cyclic GMP concentrations and inhibition of NO-synthesis by L-NAME attenuated the relaxant response to 5-HT, ergotamine and DHE. It was assumed, therefore, that the ergot derivatives stimulated the same receptor as 5-HT for induction of relaxation. This was supported by the calculated pA_2- and $-\log IC_{50}$ values of the antagonists against (±)-α-methyl-5-HT and ergotamine which did not differ significantly.

Vascular effects of 5-HT agonists are mediated through various receptor subtypes. It is conceivable that the relaxant responses to ergotamine and DHE might be due to stimulation of $5\text{-}HT_{1D}$ receptors since in porcine coronary arteries 5-HT-induced endothelium-dependent relaxation has been found to be mediated through $5\text{-}HT_{1D}$ receptors (Molderings *et al.*, 1989; Schoeffter & Hoyer, 1990). Furthermore, in calf substantia nigra sumatriptan, ergotamine and DHE are also potent agonists at $5\text{-}HT_{1D}$ receptors (Hoyer & Schoeffter, 1991). High affinity for human recombinant $5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ receptors has been found for 5-CT, ergotamine and DHE (Peroutka, 1994). However, the potencies of 5-HT agonists in porcine pulmonary arteries differed from those obtained for tissues with $5\text{-}HT_{1D}$-receptors. While in porcine pulmonary arteries 5-HT and (±)-α-methyl-5-HT were at least ten times more potent than 5-CT the latter is the most potent agonist at $5\text{-}HT_{1D}$ binding sites. Therefore, the involvement of $5\text{-}HT_{1D}$ or $5\text{-}HT_1$-like receptors can be excluded. The same applies for the $5\text{-}HT_{2A}$ receptor since ketanserin, even at high concentrations, was only a weak inhibitor. On the other hand, pizotifen which has a low affinity for $5\text{-}HT_{1D}$ but high affinity for $5\text{-}HT_{2C}$ binding sites in mammalian brain membranes (Hoyer, 1989), was a potent antagonist against ergotamine and (±)-α-methyl-5-HT in the present study.

The endothelial 5-HT receptors from various vessels and different species are presently classified as 'atypical', but they, nevertheless, clearly display the pharmacology of $5\text{-}HT_2$-like receptors (Martin, 1994). Data from our previous studies suggest that the endothelial 5-HT-receptors in porcine pulmonary arteries are similar to the $5\text{-}HT_{2B}/5\text{-}HT_{2C}$ receptor subtypes (Glusa & Richter, 1993). Several reports on cloning, distribution and pharmacology of 5-HT receptors have provided evidence that the $5\text{-}HT_{2B}$ receptor has a widespread distribution in peripheral tissues. While in the central nervous system predominantly $5\text{-}HT_{2C}$ receptors could be demonstrated, only lower levels of expression of $5\text{-}HT_{2B}$ receptors were detected in cerebral tissue (Choi *et al.*, 1994; Schmuck *et*

al., 1994; Bonhaus *et al.*, 1995; Ullmer *et al.*, 1995). However, there is a close pharmacological similarity between 5-HT_{2B} and 5-HT_{2C} receptors (Baxter *et al.*, 1995). In most cases the potencies of antagonists tested in porcine pulmonary arteries corresponded to their affinities for 5-HT_{2B} receptors in other tissues (Foguet *et al.*, 1992; Choi *et al.*, 1994; Bonhaus *et al.*, 1995). Recent studies on the endothelium-dependent relaxation in rat jugular vein are consistent with the activation of 5-HT_{2B} receptors which are responsible for relaxation of this vessel (Ellis *et al.*, 1995). The pharmacological profile of the 5-HT_{2B} receptor fits best with the endothelial receptors which have been classified as 'atypical' or 5-HT_{2C}-like receptors (Bodelsson *et al.*, 1993; Martin, 1994; Ullmer *et al.*, 1995). It might be possible that the pharmacological differences between various atypical endothelial receptors reflect only species variations in the pharmacology of 5-HT_{2B} receptors (Ellis *et al.*, 1995).

Recently, the molecular analysis of 5-HT receptor mRNA expression revealed the expression of four 5-HT receptor mRNAs in blood vessels (Ullmer *et al.*, 1995). In porcine pulmonary and coronary arteries different mRNAs for 5-$HT_{1D\beta}$, 5-HT_{2A}, 5-HT_{2B} and 5-ht_7 receptors have been demonstrated, but there was no evidence for 5-HT_{2C} receptors. A similar distribution of the receptor subtypes was found in the aorta, renal artery, femoral vein and vena cava from rats, but the relative abundance of these mRNAs varied for different vessel types.

In summary, the present functional studies suggest that the relaxant responses of precontracted porcine pulmonary arteries to ergot derivatives and 5-HT agonists are mediated through endothelial 5-HT-receptors which might be assigned to the 5-HT_{2B} receptor subtypes.

To our knowledge, this is the first demonstration of antimigraine ergot derivatives acting as relaxant agonists at endothelial 5-HT receptors. Migraine attacks are preceded by increased plasma 5-HT levels associated with an opening of arteriovenous anastomoses in the carotid vascular bed (Ferrari & Saxena, 1993). It remains to be demonstrated in human vessels whether the well known therapeutic effectiveness of ergot derivatives in migraine therapy is due partially to an action at endothelial 5-HT_{2B} receptors.

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